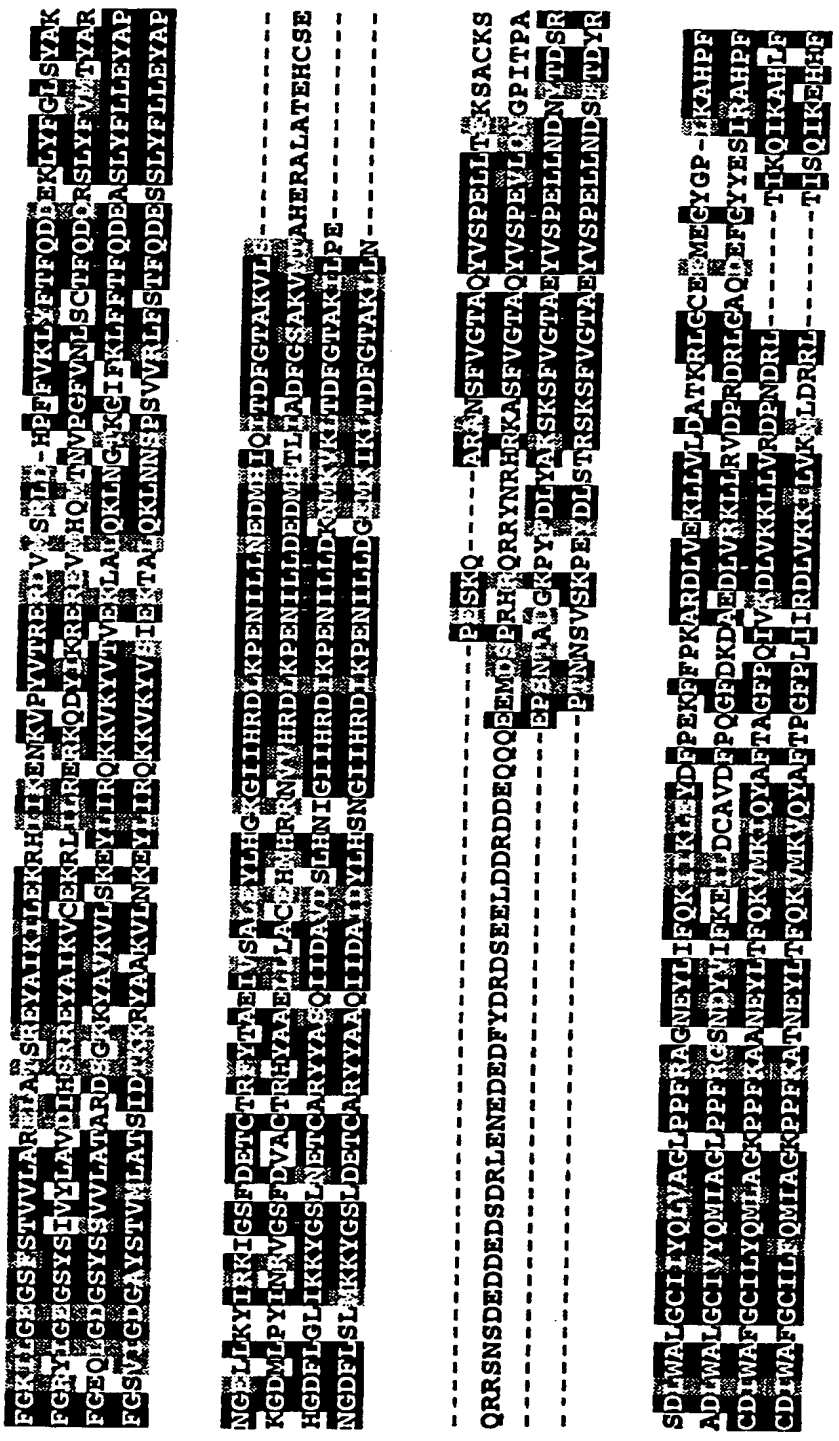


Fig. 1a

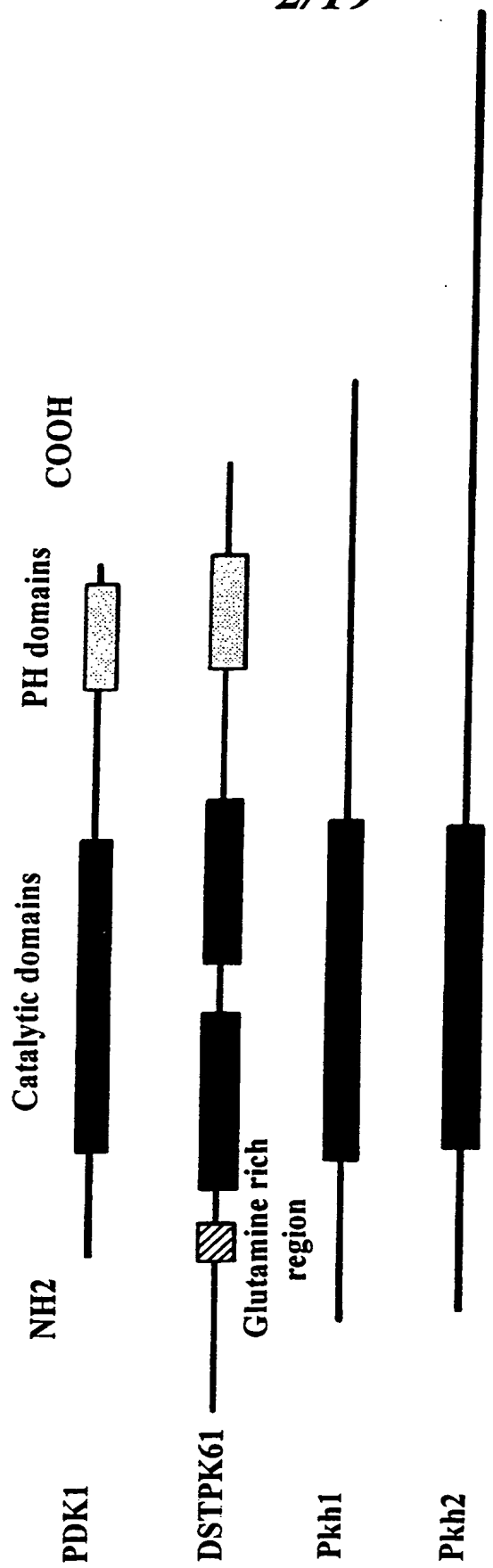
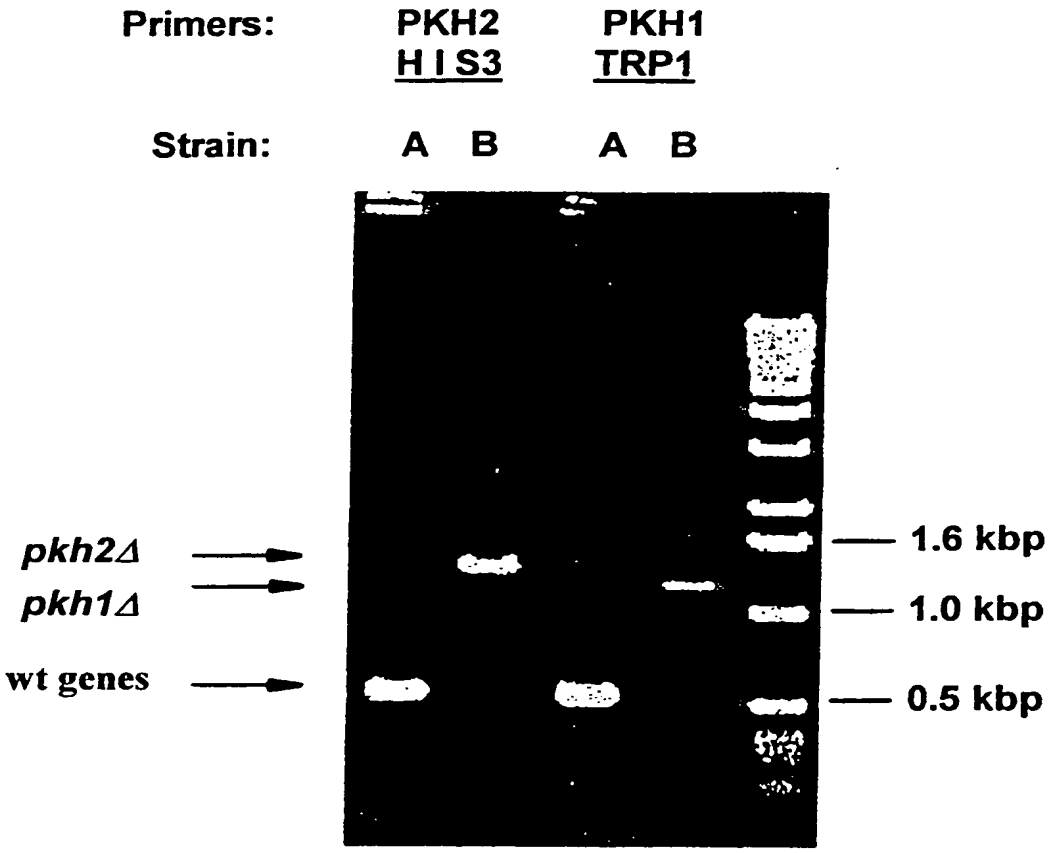

COOH
PH domains
Catalytic domains
NH2
Glutamine rich region
PDK1
DSTPK61
Pkh1
Pkh2

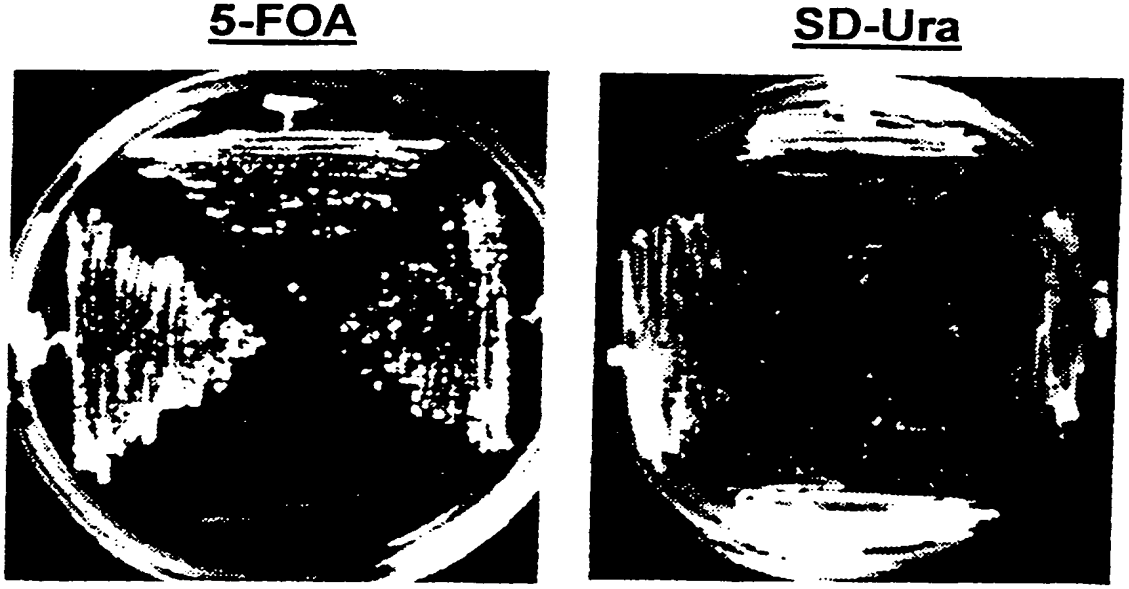
Fig. 1b

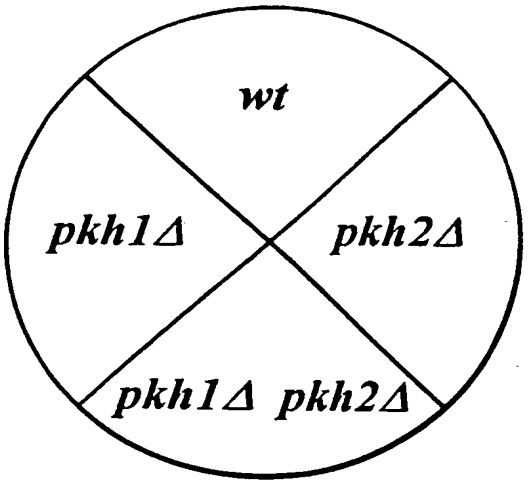


Strain A: wt [pYES2-*PKH1*]

Strain B: *pkh1Δ pkh2Δ* [pYES2-*PKH1*]

Fig. 2A





+pYES2-*PKH1*

Fig. 2B

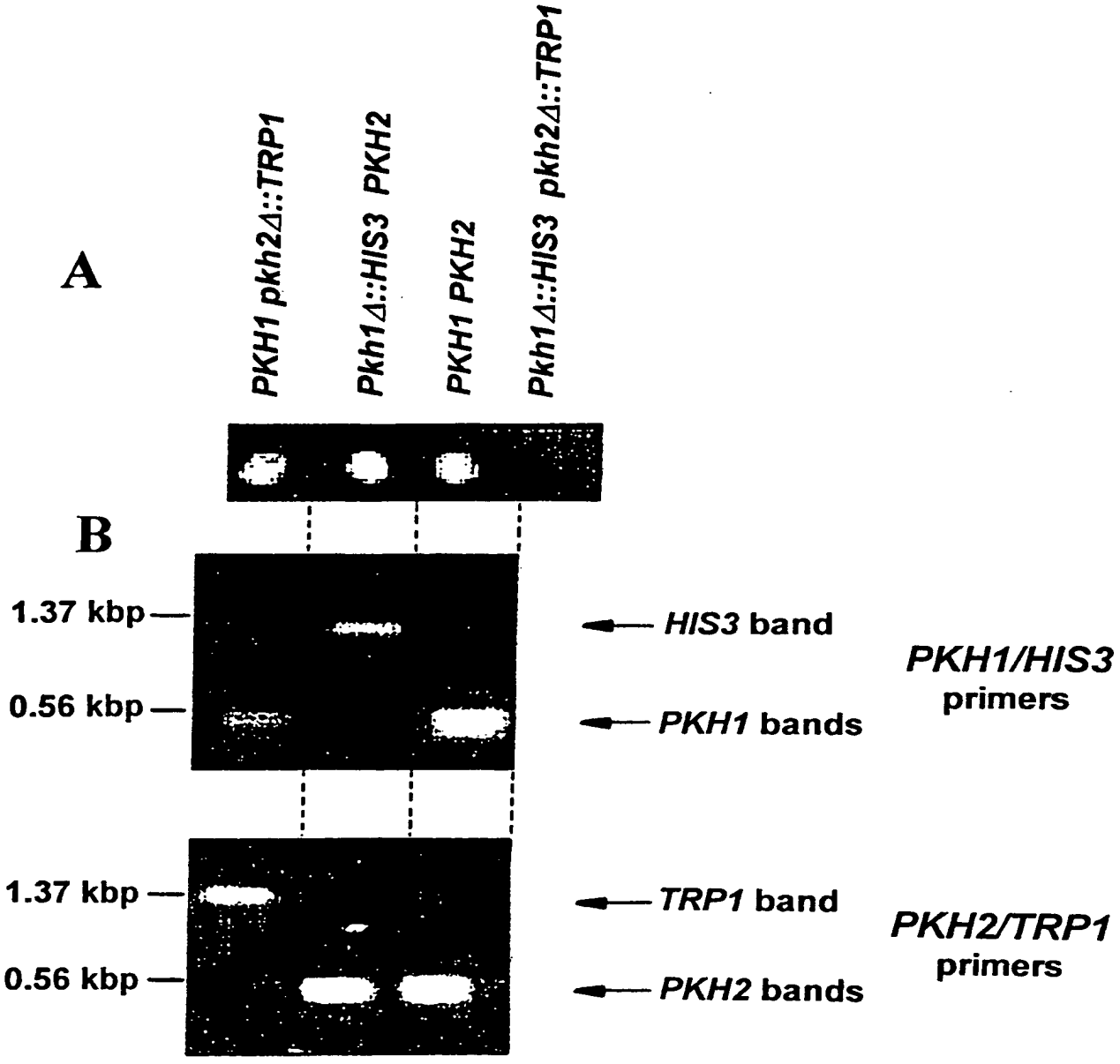
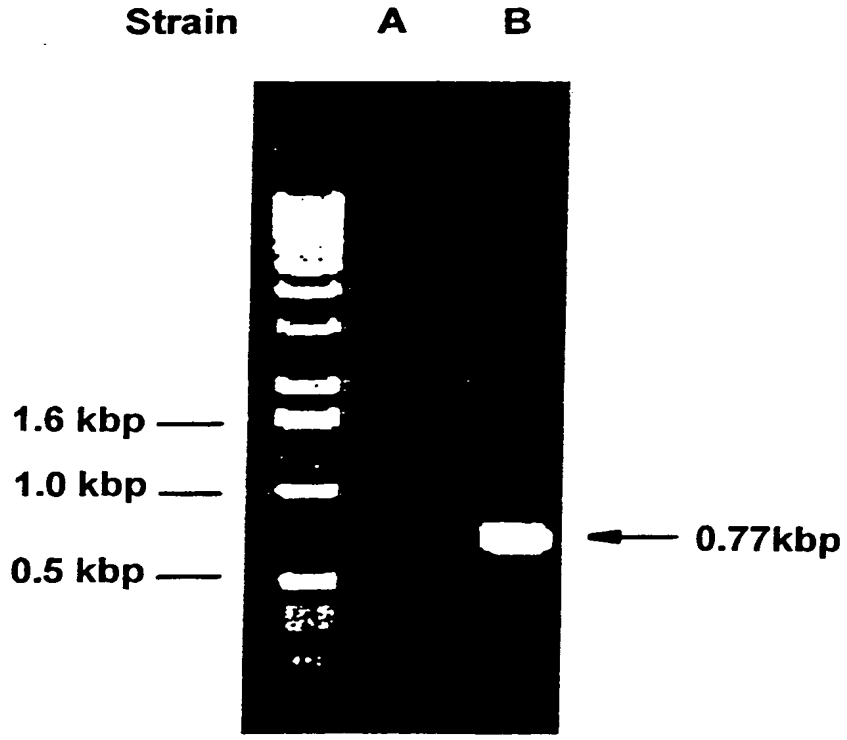

A
PKH1 pkh2Δ::TRP1
Pkh1Δ::HIS3 PKH2
PKH1 PKH2
Pkh1Δ::HIS3 pkh2Δ::TRP1
B
1.37 kbp
0.56 kbp
HIS3 band
PKH1 bands
PKH1/HIS3 primers
1.37 kbp
0.56 kbp
TRP1 band
PKH2 bands
PKH2/TRP1 primers

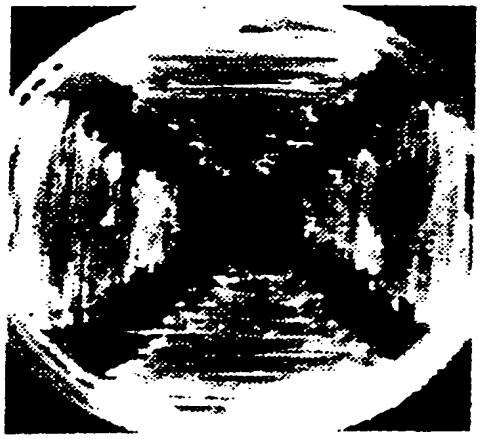
Fig. 3



Strain A: *pkh1Δ pkh2Δ* [YEplac195-*PKH1*]
Strain B: *pkh1Δ pkh2Δ* [YEplac195-PDK1]

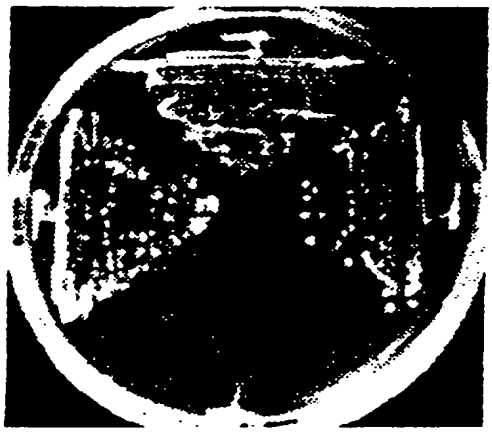
Fig. 4A

SD-Ura

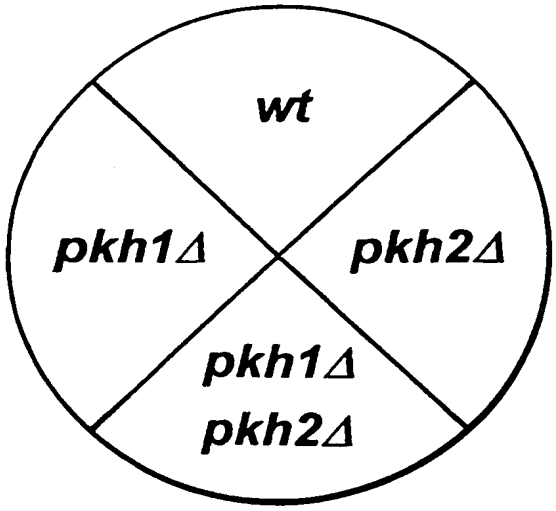


[YEplac195-PDK1]
or
[YEplac195-ΔPH-PDK1]

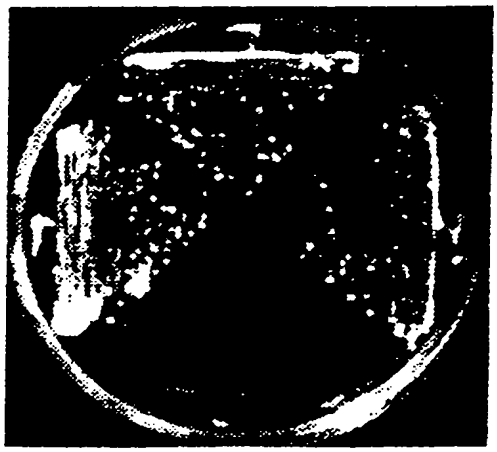
5-FOA



[YEplac195-PDK1]



5-FOA



[YEplac195-DPH-PDK1]

Fig. 4B

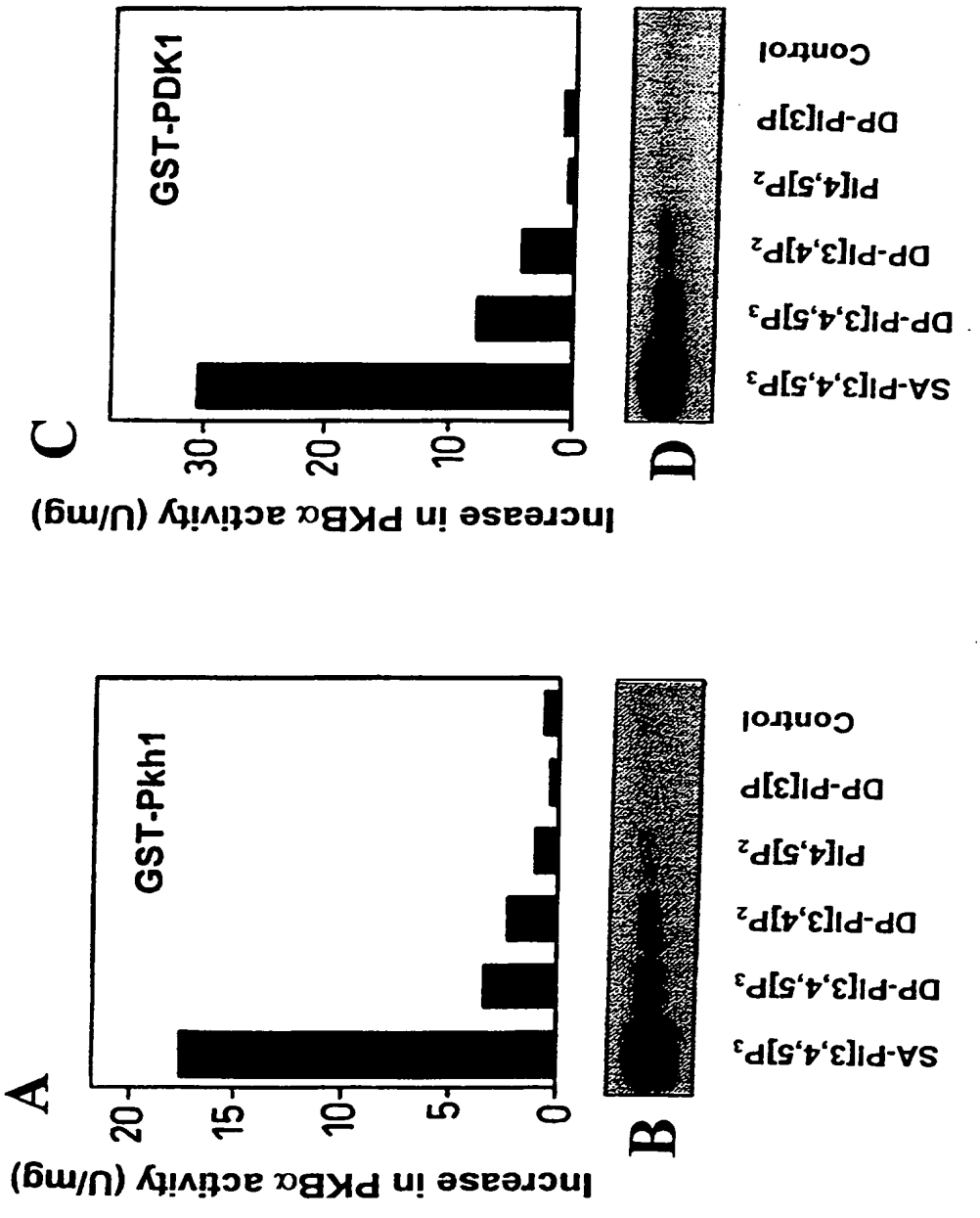

A
GST-Pkh1
Increase in PKBα activity (U/mg)
0
5
10
15
20
B
SA-PI[3,4,5]P3
DP-PI[3,4,5]P3
DP-PI[3,4]P2
PI[4,5]P2
DP-PI[3]P
Control
C
GST-PDK1
Increase in PKBα activity (U/mg)
0
10
20
30
D
SA-PI[3,4,5]P3
DP-PI[3,4,5]P3
DP-PI[3,4]P2
PI[4,5]P2
DP-PI[3]P
Control

Fig. 5

Human enzymes	Residue	Amino Acid Sequences	Residue
PKBα	303	GATMK**T**FCGTPEYLAPE.........FPQF**S**YSAS	477
SGK	251	NSTTS**T**FCGTPEYLAPE.........FLGF**S**YAPP	426
p70S6Kα	224	GTVTH**T**FCGTIEYMAPE.........FLGF**T**YVAP	393
PKCζ	533	DAKTN**T**FCGTPDYIAPE.........FRNF**S**FMNP	699
Yeast enzymes			
Ypk1	499	DDKTD**T**FCGTPEYLAPE.........FGGW**T**YVG-	665
Ykr2	496	NDKTD**T**FCGTPEYLAPE.........FGGW**T**YIG-	662
Pkc1	978	GNRTS**T**FCGTPEFMAPE.........FRGF**S**FMPD	1147
Sch9	565	KDRTN**T**FCGTTEYLAPE.........FAGF**T**FVDE	741

PDK1 phosphorylation site

PDK2 phosphorylation site

Fig. 6A

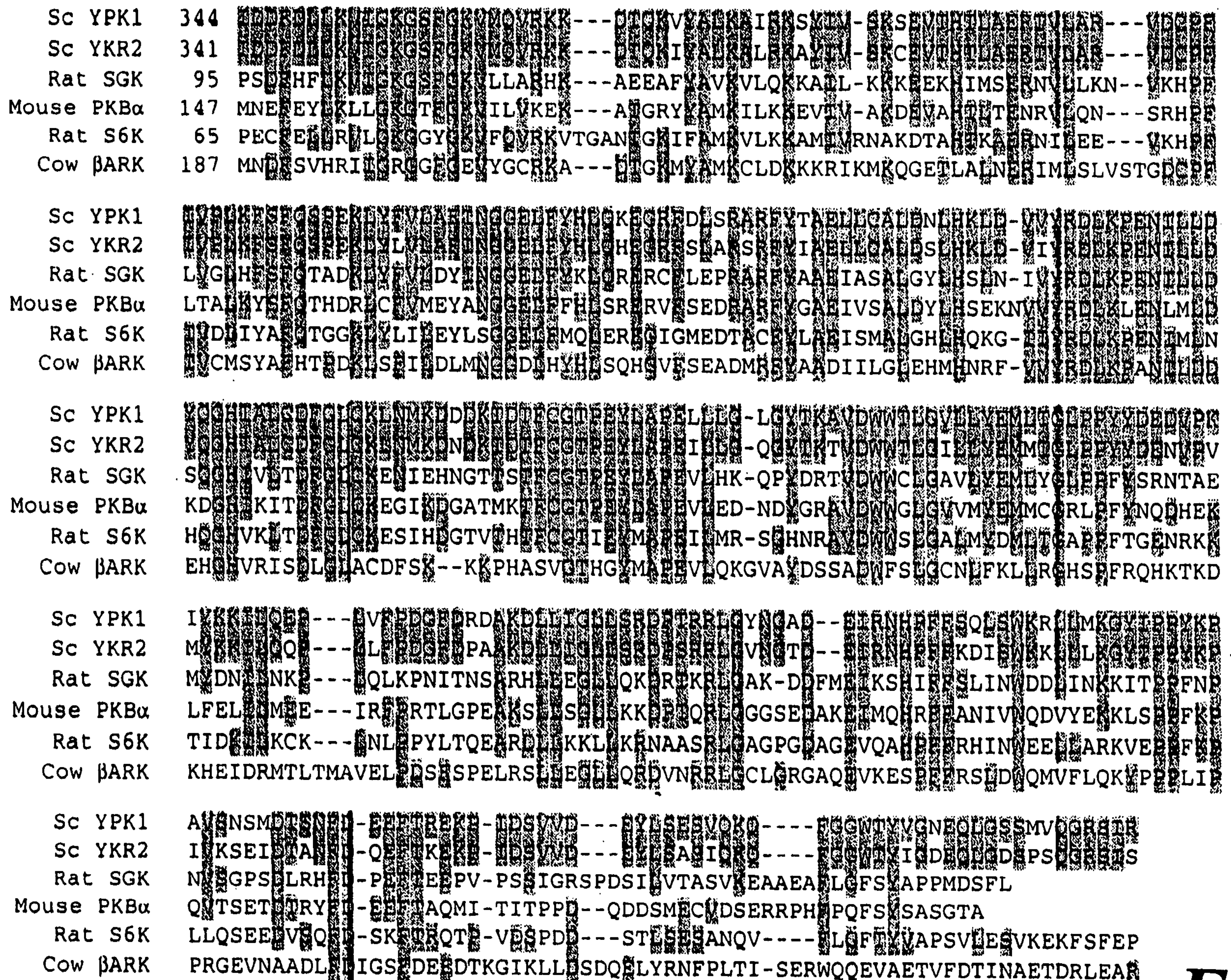
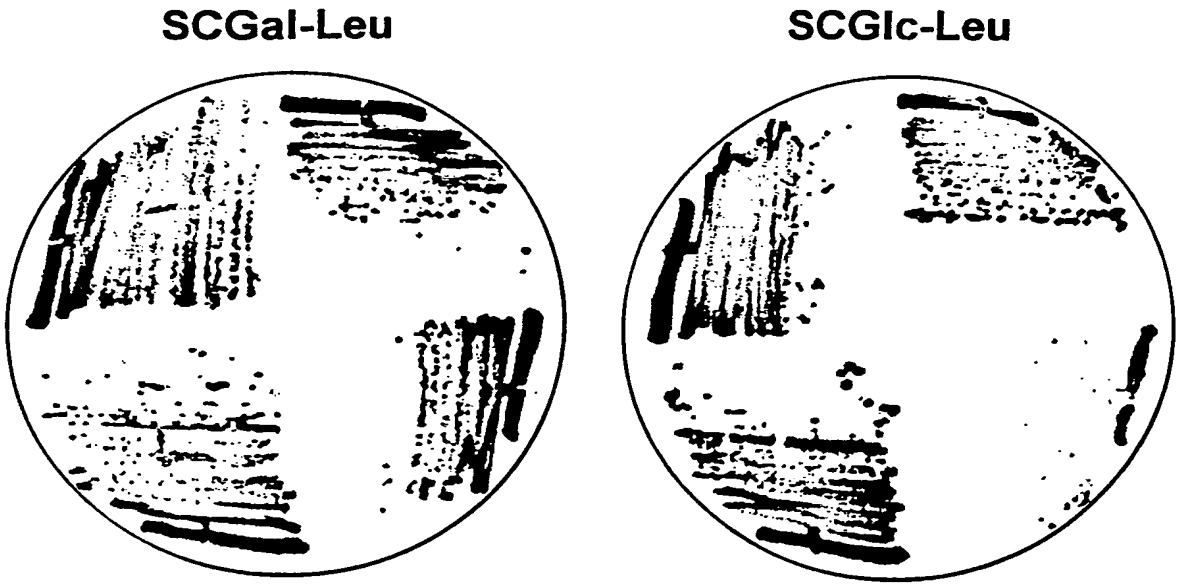
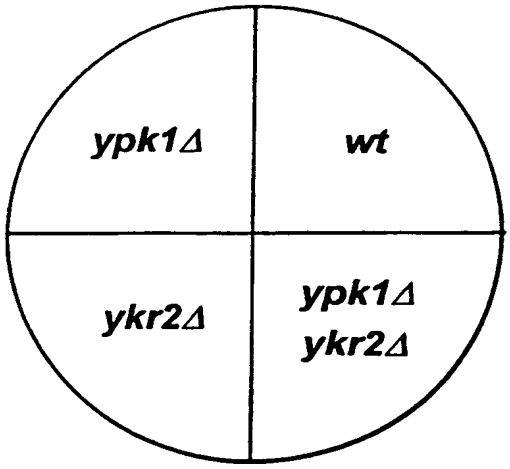


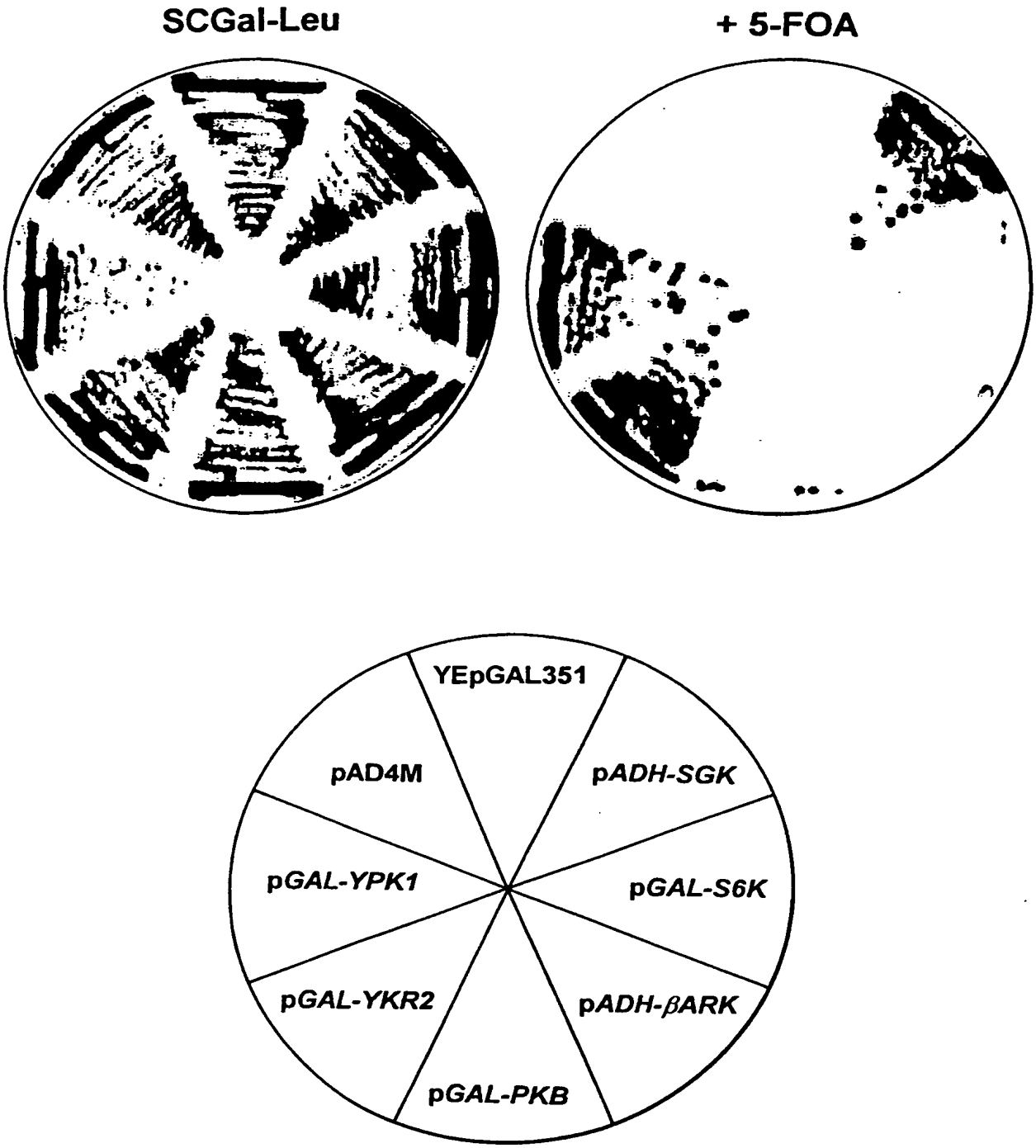
Fig. 6B





[p*GAL-YKR2*]

Fig. 7



ypk1Δ ykr2Δ [pYKR2 (URA3)]

Fig. 8A

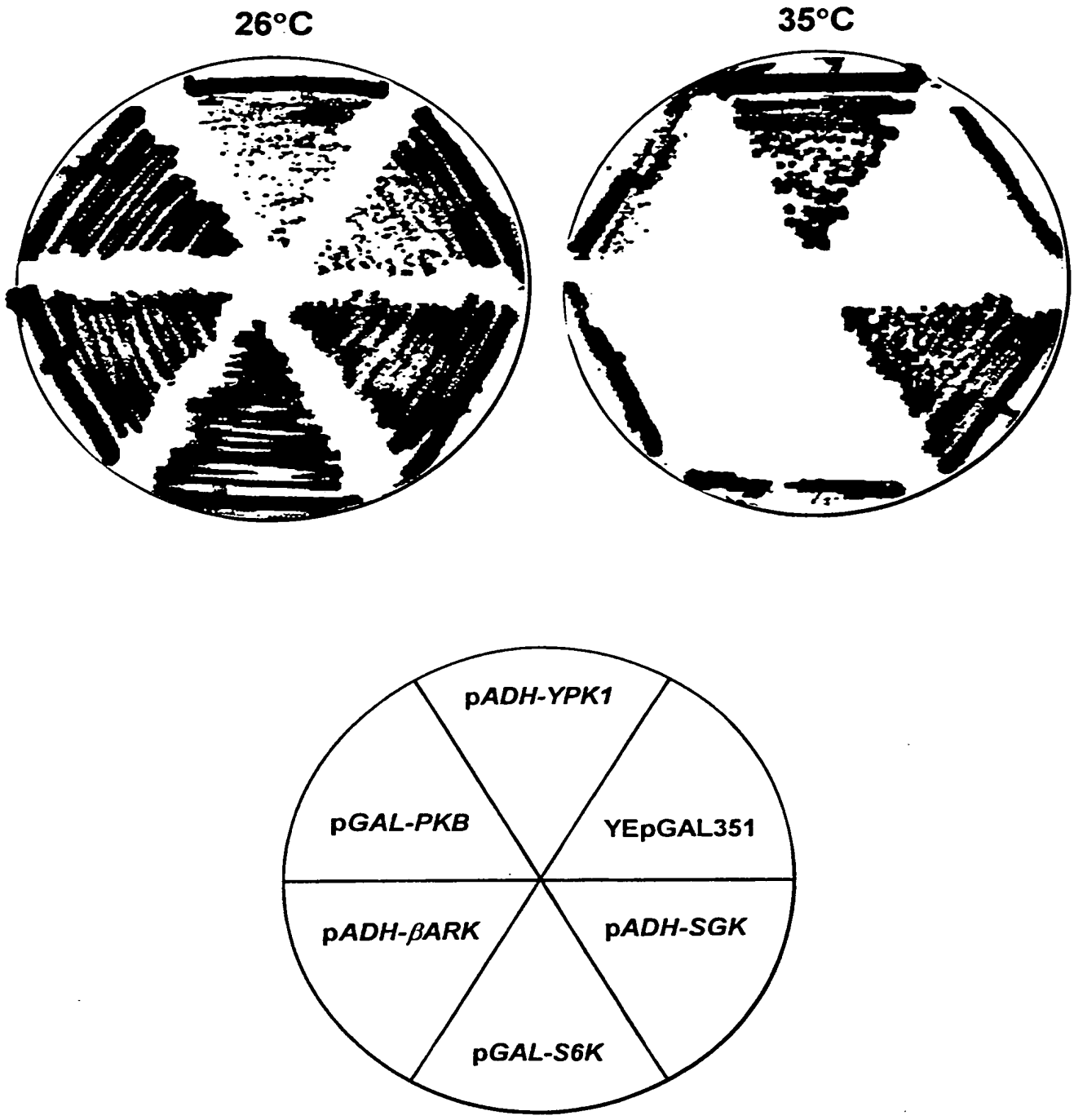

26°C
35°C
pADH-YPK1
YEpGAL351
pADH-βARK
pADH-SGK
pGAL-S6K
pGAL-PKB
ypkk1-1 ykr2Δ

Fig. 8B

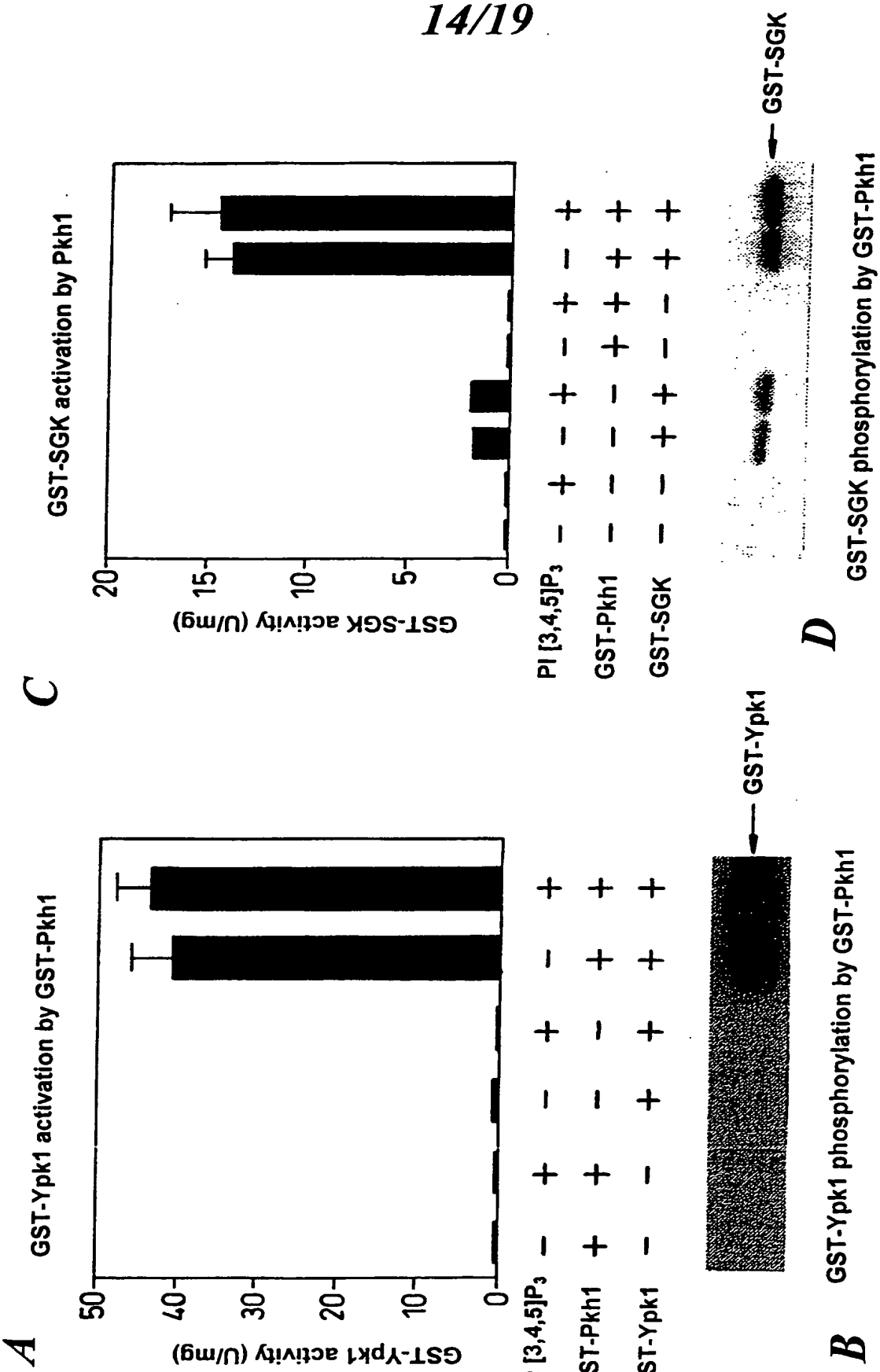

A
GST-Ypk1 activation by GST-Pkh1
GST-Ypk1 activity (U/mg)
0
10
20
30
40
50
PI [3,4,5]P3 – + – + + +
GST-Pkh1 + + – – + +
GST-Ypk1 – – + + + +
B
GST-Ypk1
GST-Ypk1 phosphorylation by GST-Pkh1
C
GST-SGK activation by Pkh1
GST-SGK activity (U/mg)
0
5
10
15
20
PI [3,4,5]P3 – + – + – +
GST-Pkh1 – – + + + +
GST-SGK – + + – + +
D
GST-SGK
GST-SGK phosphorylation by GST-Pkh1

Fig. 9

A

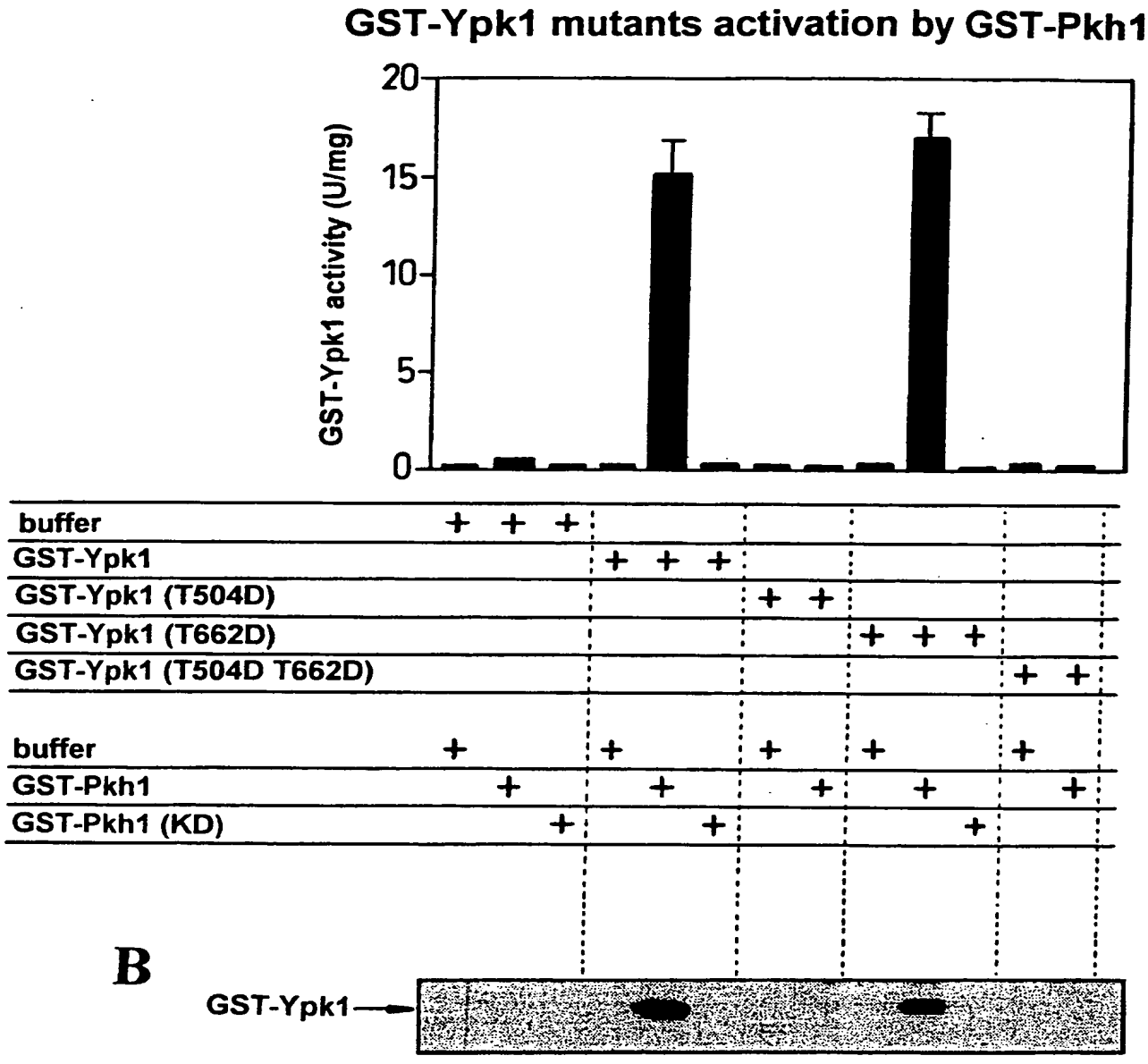

GST-Ypk1 mutants activation by GST-Pkh1
GST-Ypk1 activity (U/mg)
20
15
10
5
0
buffer
GST-Ypk1
GST-Ypk1 (T504D)
GST-Ypk1 (T662D)
GST-Ypk1 (T504D T662D)
buffer
GST-Pkh1
GST-Pkh1 (KD)
B
GST-Ypk1
GST-Ypk1 mutants phosphorylation by GST-Pkh1

Fig. 10

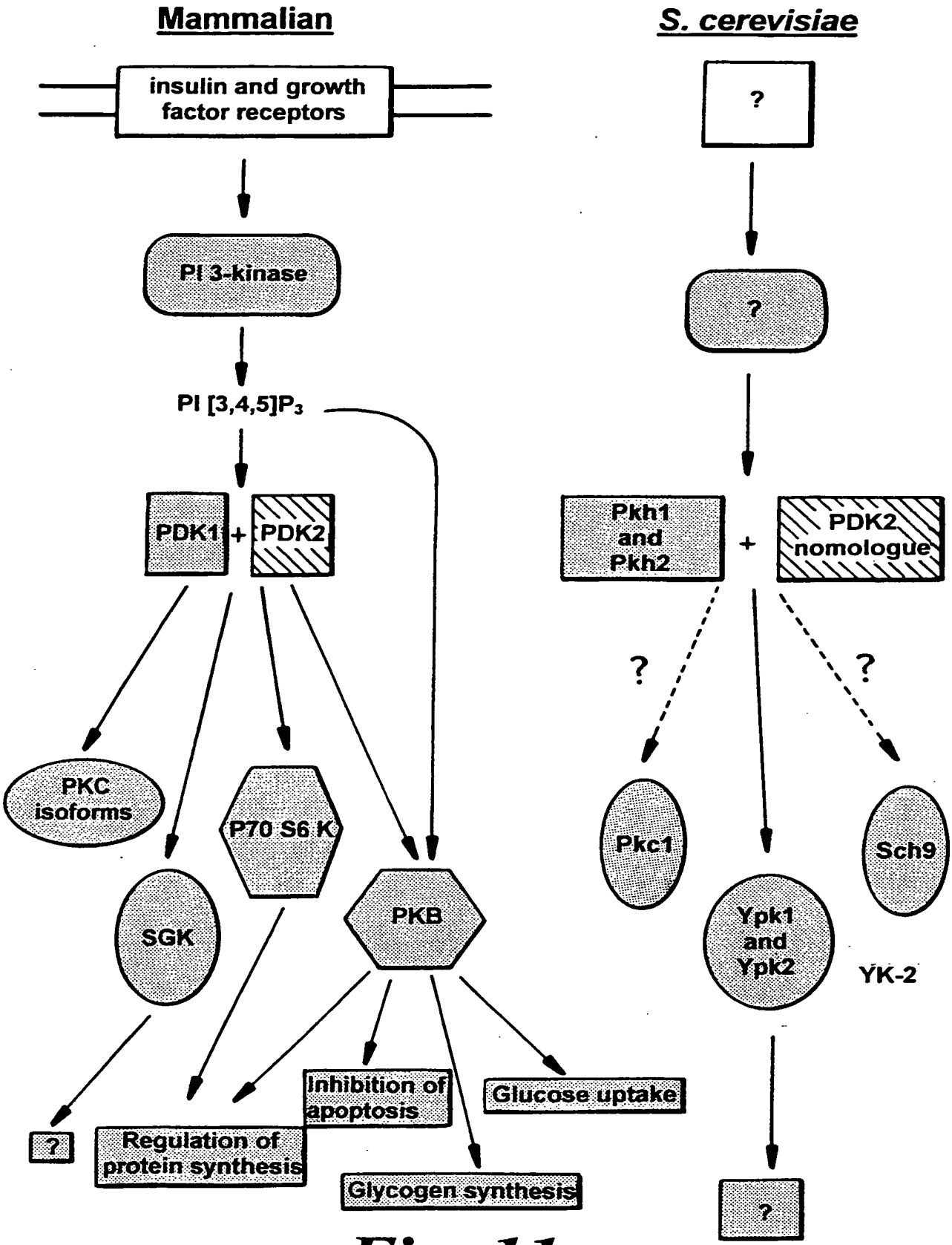

Mammalian
S. cerevisiae
insulin and growth factor receptors
?
PI 3-kinase
?
PI [3,4,5]P3
PDK1
+
PDK2
Pkh1 and Pkh2
+
PDK2 nomologue
?
?
PKC isoforms
P70 S6 K
Pkc1
Sch9
SGK
PKB
Ypk1 and Ypk2
YK-2
Inhibition of apoptosis
Glucose uptake
?
Regulation of protein synthesis
Glycogen synthesis
?

Fig. 11

CGTCAAAACCGAGGCTGCTCGAAGTACCCTCACCTACTCCAGAATGAGGGGAAT
GGTAGCAATCCTCATCGCTTTCATGAAACAGAGAAGGATGGGCCTGAACGATTT
TATTCAGAAGCTTGCCAACAACTCCTATGCATGCAAACACCCTGAAGTTCAATC
CTATTTGAAAATCTCCCAACCTCAGGAGCCCGAACTTATGAACGCCAACCCCTC
ACCTCCTCCAAGTCCCTCTCAACAAATCAACCTGGGTCCATCCTCAAATCCCCA
CGCCAAACCCTCTGACTTCCACTTCTTGAAAGTGATCGGAAAAGGCAGTTTTGG
AAAGGTTCTTCTAGCAAGGCACAAGGCAGAAGAAGCATTCTATGCCGTCAAAGT
TTTGCAGAAGAAAGCCATCTTGAAGAAGAAGGAGGAGAAGCATATTATGTCAGA
GCGCAATGTTCTGTTGAAGAATGTGAAGCACCCTTTCCTGGTGGGCCTTCACTT
CTCTTTCCAGACTGCTGACAAACTCTACTTCGTCCTAGACTACATTAATGGCGG
AGAGCTGTTCTACCATCTCCAGAGGGAGCGCTGCTTCCTGGAACCCCGTGCTCG
CTTCTACGCAGCTGAAATAGCCAGTGCCTTGGGTTATCTGCACTCCCTAAACAT
CGTTTATCGAGACTTAAAACCAGAGAATATTCTCCTAGACTCACAGGGACACAT
CGTCCTCACTGACTTTGGGCTCTGCAAGGAGAACATCGAGCACAATGGGACAAC
GTCCACCTTCTGTGGCACGCCTGAGTATCTCGCTCCTGAGGTTCTCCATAAGCA
GCCGTACGACCGGACAGTGGACTGGTGGTGCCTCGGGGCTGTCTTGTATGAGAT
GCTCTATGGCCTGCCTCCGTTCTACAGCCGGAACACAGCCGAGATGTATGACAA
TATTCTGAACAAGCCTCTCCAGCTGAAAAATATCACCAACTCAGCAAGGCACCT
GCTGGAGGGCCTCCTGCAGAAGGACCGGACCAAGAGGCTGGGTGCCAAGGATGA
CTTTATGGAGATTAAGAGTCATATTTTCTTCTCTTTGATTAACTGGGATGATCT
CATTAATAAGAAGATCACGCCCCCATTTAACCCAAATGTGAGCGGGCCCAGTGA
CCTTCGGCACTTTGATCCCGAGTTTACTGAGGAGCCGGTCCCCAGCTCCATCGG
GCGATCCCCTGACAGCATCCTTGTCACAGCCAGTGTGAAAGAAGCCGCGGAAGC
CTTCCTTGGCTTCTCCTATGCCCCTCCTATGGACTCCTTCCTCTGAAAGCTCCC
AGGATGGTTCCGAAGGATTTCCTCAGCGTTTTTCTAAAGTGTTTTAGTTAGCCT
TTGGTGGAGTTACCAGCTGACAGAACATCTTAGAAGAGAAATTTGCACACCAGG
AAGCTTGGCAGTCCCGCCTGCCGGGGCCTGCACGCGGCTTGTTGACGCGGAAGC
TTTCCGGAAGCTTTCCGAAGAGCACATCCTCCTCTCAGTGAGCTAGTGAGGTCT
TCATTTCTTTTCTTCCTTCCAACGTGGTGCTAGCTCTAAAGGAGCTTGAGAGTG
CCGCCTGAGACGCACCTTGGTCTCAGTGAGAAGGAAGATGCAGGTCTAAGAGGG
ATCTCCGCAGGTCTGAGCTGTGATCAAGAATATTCTGCAATGTGCCTTTTCTGA

Fig. 12 (part 1 of 2)

GATTGTGTTAGCTCCAAAGCTTTTCCTATCGCAGAGTGTCCAGTTTTTGTTTGT
TTTTTTTTTTTTGTTTTGTTTTTTGTTTCTTTTTTTTTCCCAACCCTTGCGTATT
TCCCATGTGTGCAGTTAGTGTGAGTGCTATGCCTGATCACAGACAGTTTTGTTG
TAAGCATCAATGTGACACTTGCAGGACACTACAATGTGGGACATTGTTTGTTTC
TTCCACATTTGGAAGATAAATTTATGCGCAGACTGTTTTTTTTTGTAAGATATA
ATAACTAAAACCTATTGAAACGGTCTTGCAGTGACGAGCATCCAGATGCTTGAG
GGAAGCATTGCTGCTACAAATATTTCTATTTTTAGAAAGGGTTTTTATGGACCA
ATGCCCCAGTTGTCAGTCAGAGCCGTTGGTGTTCATGTTAAAATGTCACCTGCA
AAATGGGCATTATTTATGGTTCCCCCCAACCTTTGTTCATTTTCTTTTGCATTC
CTGATTATTGTGTGTAAAGAAAGTCTGTACATTGGGTTATAACACTAGATATTT
AAACTTACAGGCTTATTTGTAAACCATCATTTTAATGTCCTGTAATTAACATGG
TTATAACATGTACACTCCCCCCTACTCACCACACAACTTTTTTTGTGTGCGGTG
AAACCAATTTTGGTTTGCAATAAAATCTTGAAAACTATTTGCG

Fig. 12 (part 2 of 2)

MTVKTEAARSTLTYSRMRGMVAILIAFMKQRRMGLNDFIQKLANNSYACKHPEV
QSYLKISQPQEPELMNANPSPPPSPSQQINLGPSSNPHAKPSDFHFLKVIGKGS
FGKVLLARHKAEEAFYAVKVLQKKAILKKKEEKHIMSERNVLLKNVKHPFLVGL
HFSFQTADKLYFVLDYINGGELFYHLQRERCFLEPRARFYAAEIASALGYLHSL
NIVYRDLKPENILLDSQGHIVLTDFGLCKENIEHNGTTSTFCGTPEYLAPEVLH
KQPYDRTVDWWCLGAVLYEMLYGLPPFYSRNTAEMYDNILNKPLQLKPNITNSA
RHLLEGLLQKDRTKRLGAKDDFMEIKSHIFFSLINWDDLINKKITPPFNPNVSG
PSDLRHFDPEFTEEPVPSSIGRSPDSILVTASVKEAAEAFLGFSYAPPMDSFL

Fig. 13